Toxic effect of *Thevetia peruviana* and *Alstonia scholaris* latices on the freshwater snail *Lymnaea acuminata*

Toxicidad de los látex de *Thevetia peruviana* y *Alstonia scholaris* sobre el molusco dulceacuícola *Lymnaea acuminata*

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ABSTRACT

The aqueous and partially purified latex extracts of plants *Thevetia peruviana* and *Alstonia scholaris* (Family Apocynaceae) have potent molluscicidal activity. Sub-lethal doses of aqueous and partially purified latex extracts of both the plants also significantly alter the levels of total protein, total free amino acid, nucleic acid (DNA and RNA) and the activity of enzyme protease, acid and alkaline phosphatase in nervous tissue of the snail *Lymnaea acuminata* in time and dose dependent manner. The biologically active compounds present in *Thevetia peruviana* plant are Apigenine (Flavonoid) and triterpenoid glycosides, while a number of alkaloids (i.e. pseudo-akuammigine, Betulin, Ursolic acid and ß-sitosterol etc.), steroids and triterpenoids are present in *Alstonia scholaris* plant.

RESUMEN

Los extractos de latex acuosos y parcialmente purificados de las plantas *Thevetia peruviana* y *Alstonia scholaris* (Familia Apocynaceae) tienen potente actividad molusquicida. Dosis subletales de dichos extractos alteran significativamente los niveles de proteínas totales, aminoácidos libres, ácidos nucléicos y actividad de los enzimas proteasa, fosfatasa ácida y alcalina en el tejido nervioso de *Lymnaea acuminata* de manera tiempo-dependiente y dosis-dependiente. Los componentes activos de *Thevetia peruviana* son Apigenina (Flavonoide) y glicósidos triterpenoides, mientras que en *Alstonia scholaris* aparecen un cierto número de alcaloides (i.e. pseudo-akuammigina, betulina, ácido ursolico y ß-sitosterol entre otros), esteroides y triterpenos.

KEY WORDS: *Lymnaea acuminata*, *Thevetia peruviana*, *Alstonia scholaris*, metabolism.
PALABRAS CLAVE: *Lymnaea acuminata*, *Thevetia peruviana*, *Alstonia scholaris*, metabolismo.

INTRODUCTION

The use of synthetic or petroleum based molluscicides for controlling vector snails cause serious environmental pollution (Mian and Mulla, 1992; Redinger, 1976; Susan, Veeraiah and Tilak, 1999). To overcome the problem and to search for eco-friendly molluscicides, a number of extracts and essential

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oils and their isolates have been evaluated for use as molluscicides due to their high toxicity, easy availability and easy biodegradability (MARSTON AND HOSTETTMANN, 1985; KINGHORN AND EVANS, 1975; SINGH, SINGH, MISRA AND AGARWAL, 1996). These materials have shown encouraging results for vector controlling properties with various snail species.

Thevetia peruviana and *Alstonia scholaris*, are common medicinal plants of family Apocynaceae. The latex of *Thevetia peruviana* is used in teeth cavities for relief from toothache and the latex of *Alstonia scholaris* is applied to ulcer, sores, tumers and rheumatic pain and is used for curing toothache (RAMA RAO, 1967). Both the plants have potent molluscicidal and anti-cholinesterase activity against harmful snails and slugs (PANIGRAHI AND RAUT, 1994; SINGH, YADAV AND SINGH, 2000; SINGH AND SINGH, 2002). The mechanism by which the active compounds present in both the plants causes snail death is not known.

The toxicological actions of *Thevetia peruviana*, may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides (VOIGTLANDER AND BALSAM, 1970) while a number of alkaloids (pseudo-akuammigine in addition to betulin, ursolic acid and ß-sitosterol), steroids and triterpenoids are present in *Alstonia scholaris* (BANERJI AND BANERJI, 1977).

We are interested in knowing the mode of action and long-term effect of these plant products on snails, because these substances cannot be put to commercial use without a study of these aspects as well. In the present study, the effect of sub-lethal doses of aqueous and partially purified latex extracts of *Thevetia peruviana* and *Alstonia scholaris* is examined on nitrogenous metabolism of freshwater snail *Lymnaea acuminata*. This snail is intermediate host of liver fluke *Fasciola hepatica* and *Fasciola gigantica*, which causes endemic fascioliasis in cattle and live- stock in northern parts of India (SINGH AND AGARWAL, 1981).

MATERIALS AND METHODS

Latex of *Thevetia peruviana* and *Alstonia scholaris* were collected from Botanical garden of D.D.U. Gorakhpur University, Gorakhpur, India. White latex produced by these plants was drained into glass tubes fallowing cutting of the stem apices and lyophilised at –40°C and the lyophilised dry powder was used for further study. The wet weight of one ml of latex of *Thevetia peruviana* and *Alstonia scholaris* was 820 mg and 1000 mg and dry weight was 340 mg and 400 mg, respectively.

For aqueous extracts: The freeze-dried powder was mixed with appropriate volume of distilled water to obtain the desired concentrations.

For partial purification: The lyophilised latex powder was extracted sequentially with 5 mL each of chloroform → carbon tetrachloride → acetone → diethyl ether → ethyl alcohol. Centrifugation for 20 min at 2000 g was carried out in a refrigerated centrifuge, at -4°C, after each extraction. The solvent fraction was decanted and the solvent allowed to evaporates. The dried up soluble fraction was re-dissolved in water for further experiments.

Adult snail *Lymnaea acuminata* (2.6±0.3 cm in shell height) was collected locally and used as test animals. The snails were maintained and treated with aqueous and partially purified extracts of latices of the test plants according to SINGH AND AGARWAL (1990). Adult *Lymnaea acuminata* were kept in glass aquaria containing 3 litres of de-chlorinated tap water. Each aquarium contained 20 experimental animals.

Treatment protocol for dose- response relation

Snails were exposed for 24h or 96h to 40% and 80% of LC_{50} doses of *Thevetia peruviana* and *Alstonia scholaris* latices. LC_{50} doses were determined in an earlier study by SINGH (2000). The 24h or 96h doses for *Thevetia peruviana* were, respectively, 0.43 mg/L and 0.17 mg/L and for *Alstonia scholaris* were 4.76 mg/L and 1.76 mg/L.

After completion of treatment the test animals were removed from the aquaria and washed with water. The nervous tissue of *Lymnaea acuminata* was excised and used for biochemical analysis. Control animals were held in similar conditions without any treatment. Each experiment was replicated at least six times and the values have been expressed as mean ±SE of six replicates. Student's 't' test and analysis of variance were applied to locate significant changes (SOKAL AND ROHLF, 1973).

Biochemical estimation

Protein: Protein levels were estimated according to the method of LOWRY, ROSEBROUGH, FARR AND RANDALL (1951) using bovine serum albumin as standard. Homogenates (5 mg/mL, w/v) were prepared in 10% Trichloroacetic acid (TCA).

Total free amino acids: Estimation of total free amino acid was made according to the method of SPICES (1957). Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at 6000 g and used for amino acid estimation.

Nucleic acids: Estimation of nucleic acid (DNA and RNA) was performed, by the methods of SCHNEIDER (1957) using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/mL, w/v) were prepared in 5% TCA at 90°C, centrifuged at 5000 g for 20 min and supernatant was used for estimation of nucleic acids.

Protease: Protease activity was estimated by the method of MOORE AND STEIN (1954). Homogenate (50 mg/mL, w/v) was prepared in cold distilled water. Optical density was measured at 570 nm. The enzyme activity was expressed in m moles of tyrosine equivalent/mg protein/h.

Acid and alkaline phosphatase: The activity of acid and alkaline phosphatase in the nervous tissue was determined, according to the method of ANDERSCH AND SZCYPINSKI (1947) as modified by BERGMEYER (1967) and SINGH AND AGARWAL (1983). Tissue homogenates (2% w/v) were prepared in ice-cold 0.9% sodium chloride solution and centrifuged at 5000 g at (0°C) for 15 min. Optical density was measured at 420 nm against a blank, prepared simultaneously. The enzyme activity has been expressed as amount of ρ-nitrophenol formed/30min/mg protein in supernatant.

RESULTS

Data of sub-lethal (40% and 80% of LC_{50}) exposure of freshwater snail *Lymnaea acuminata* against aqueous and serially extracted latex of *Thevetia peruviana* and *Alstonia scholaris* for 24h and 96h on nitrogenous metabolism in nervous tissue *are* given in Table I-IV.

Thevetia peruviana

Exposure of snails to 40% and 80% of LC_{50} of aqueous latex extracts of *Thevetia peruviana* for 24h or 96h caused significant alterations in nitrogenous metabolism in nervous tissue of the freshwater snail *Lymnaea acuminata* (Table I-II). Total protein levels were reduced to 54% and 38% of controls, respectively after exposure to 40% and 80% of LC_{50} (24h) of aqueous latex extract. The maximum decrease in protein level (30 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. DNA level was reduced to 81% and 71% of controls after treatment with 40% and 80% of LC_{50} (24h), respectively. The maximum decrease in DNA (35 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. RNA level was reduced to 88% and 70% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue of *Lymnaea acuminata*. The maximum decrease in RNA (42 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. Total free amino acid levels were induced to 149% and 166% of controls after treatment with 40% and 80% of LC_{50} (96h) of aqueous latex extracts respectively in nervous tissue of snail *Lymnaea acuminata* (Table I-II).

Table I. Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg moles of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in nervous tissue of *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous and partially purified latex extracts of *Thevetia peruviana* for 24 h.

Tabla I. Cambios en los niveles de proteína total, aminoácidos libres, ácidos nucléicos (DNA y RNA) (µg/mg), proteasa (µg moles de equivalentes de tirosina/mg proteína/h) y fosfatasas ácida y alcalina (µ moles sustrato hidrolizado/30 min/mg proteína) en el tejido nervioso de Lymnaea acuminata *tras 24 h de exposición a extractos acuosos y parcialmente purificados de latex de* Thevetia peruviana *al 40% y 80% de* LC_{50}.

	Nature of latex	Control	40% of LC50	80% of LC50
Protein	A	65.00±0.28 (100)	34.80±0.36+ (54)	24.50±0.38+ (38)
	B	65.30±0.78 (100)	35.50±0.68+ (54)	27.50±0.84+ (42)
Amino acid	A	34.60±0.36 (100)	48.50±0.68+ (140)	45.60±0.76+ (152)
	B	34.40±0.65 (100)	51.20±0.77+ (137)	47.50±1.02+ (146)
DNA	A	75.40±1.02 (100)	60.90±0.88+ (81)	53.70±0.70+ (71)
	B	75.60±1.12 (100)	62.80±1.02+ (83)	55.50±1.05+ (73)
RNA	A	60.10±0.52 (100)	52.80±0.60+ (88)	42.06±0.52+ (70)
	B	61.20±0.82 (100)	54.60±0.70+ (88)	44.05±0.96+ (72)
Protease	A	0.325±0.067 (100)	0.378±0.052+ (116)	0.420±0.048+ (129)
	B	0.325±0.046 (100)	0.375±0.062+ (115)	0.415±0.062+ (128)
Acid phosphatase	A	0.193±0.0005 (100)	0.171±0.0004+ (89)	0.148±0.0003+ (77)
	B	0.193±0.0010 (100)	0.178±0.0003+ (92)	0.152±0.0006+ (79)
Alkaline phosphatase	A	0.381±0.0015 (100)	0.338±0.0006+ (89)	0.251±0.0005+ (66)
	B	0.382±0.0012 (100)	0.344±0.0005+ (90)	0.254±0.0003+ (66)

Values are mean ±SE of six replicates
Values in parenthesis are % change with control taken as 100%
+, Significant (P<0.05) student's 't' test was applied between control and treated groups
A – Supernatant of aqueous solution of latex
B – Latex serially extracted through chloroform, carbon tetrachloride, acetone, diethyl ether and ethyl alcohol

Activity of acid phosphatase was inhibited to 89% and 77% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue of snail. Activity of alkaline phosphatase was reduced to 89% and 66% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue of snail. The maximum decrease in acid and alkaline phosphatase 37 % and 45% of controls, respectively, was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. Protease activity was increased to 116% and 129% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in the nervous tissue of snail *Lymnaea acuminata*. The maximum increase in protease activity (141% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract (Table I-II).

Latex was sequentially extracted with organic solvents also caused a similar alteration in nitrogenous metabolism of snail. Alterations caused by

Table II. Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg moles of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in nervous tissue of *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous and partially purified latex extracts of *Thevetia peruviana* for 96 h.

Tabla II. Cambios en los niveles de proteína total, aminoácidos libres, ácidos nucléicos (DNA y RNA) (µg/mg), proteasa (µg moles de equivalentes de tirosina/mg proteína/h) y fosfatasas ácida y alcalina (µ moles sustrato hidrolizado/30 min/mg proteína) en el tejido nervioso de Lymnaea acuminata *tras 96 h de exposición a extractos acuosos y parcialmente purificados de latex de* Thevetia peruviana *al 40% y 80% de* LC_{50}.

	Nature of latex	Control	40% of LC50	80% of LC50
Protein	A	68.50±0.48 (100)	30.50±0.44⁺ (44)	20.80±0.44⁺ (30)
	B	68.40±0.76 (100)	31.60±0.72⁺ (46)	21.60±0.48⁺ (32)
Amino acid	A	35.30±0.98 (100)	52.50±0.26⁺ (149)	58.60±0.36⁺ (166)
	B	35.30±0.98 (100)	40.50±0.46⁺ (143)	56.70±0.78⁺ (161)
DNA	A	72.16±0.93 (100)	39.50±0.46⁺ (55)	25.20±0.39⁺ (35)
	B	72.20±0.05 (100)	40.40±0.78⁺ (56)	28.30±0.98⁺ (39)
RNA	A	62.50±1.02 (100)	36.40±0.45⁺ (58)	26.45±0.78⁺ (42)
	B	62.80±1.12 (100)	38.50±0.78⁺ (61)	28.35±0.98⁺ (45)
Protease	A	0.345±0.058 (100)	0.420±0.072⁺ (122)	0.186±0.025⁺ (141)
	B	0.346±0.068 (100)	0.418±0.075⁺ (121)	0.128±0.032⁺ (138)
Acid phosphatase	A	0.192±0.0003 (100)	0.096±0.0005⁺ (50)	0.071±0.0006⁺ (37)
	B	0.191±0.0004 (100)	0.101±0.0007⁺ (53)	0.074±0.0011⁺ (39)
Alkoline phosphatase	A	0.400±0.0007 (100)	0.187±0.0010⁺ (47)	0.181±0.0007⁺ (45)
	B	0.390±0.0015 (100)	0.194±0.0004⁺ (50)	0.184±0.0004⁺ (47)

Details are as given in Table I

sequentially extracted latex, though statistically in significant, appeared lower than the water extracted latex at all the doses and exposure periods (Table I-II).

Alstonia scholaris

Exposure of snails to 40% and 80% of LC_{50} of aqueous latex extracts of *Alstonia scholaris* for 24h or 96h caused significant alterations in nitrogenous metabolism in nervous tissue of the freshwater snail *Lymnaea acuminata* (Table III-IV). Total protein levels were reduced to 55% and 40% of controls, respectively after exposure to 40% and 80% of LC_{50} (24h) of aqueous latex extract. The maximum decrease in protein level (35 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. DNA level was reduced to 85% and 75% of controls after treatment with 40% and 80% of LC_{50} (24h), respectively. The maximum decrease in DNA (39 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. RNA level was reduced to 90% and 75% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue of *Lymnaea acuminata*. The maximum decrease in RNA (45 % of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract. Total free amino acid levels were induced to 145% and 160% of controls after treatment with 40% and 80% of LC_{50} (96h) of aqueous latex extracts respectively in nervous tissue of snail (Table III-IV).

Table III. Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg moles of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in nervous tissue of *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous and partially purified latex extracts of *Alstonia scholaris* for 24 h.

Tabla III. Cambios en los niveles de proteína total, aminoácidos libres, ácidos nucléicos (DNA y RNA) (µg/mg), proteasa (µg moles de equivalentes de tirosina/mg proteína/h) y fosfatasas ácida y alcalina (µ moles sustrato hidrolizado/30 min/mg proteína) en el tejido nervioso de Lymnaea acuminata *tras 24 h de exposición a extractos acuosos y parcialmente purificados de latex de* Alstonia scholaris *al 40% y 80% de* LC_{50}.

	Nature of latex	Control	40% of LC50	80% of LC50
Protein	A	66.20±0.56 (100)	36.50±0.76$^+$ (55)	26.60±0.98$^+$ (40)
	B	66.50±0.78 (100)	38.20±0.56$^+$ (57)	29.90±0.78$^+$ (45)
Amino acid	A	35.50±0.57 (100)	47.90±0.78$^+$ (135)	52.50±0.68$^+$ (148)
	B	36.20±0.56 (100)	47.80±1.05$^+$ (132)	52.50±0.68$^+$ (145)
DNA	A	74.80±1.05 (100)	63.60±0.54$^+$ (85)	56.10±0.58$^+$ (75)
	B	75.20±1.06 (100)	65.40±0.96$^+$ (87)	57.20±0.46$^+$ (76)
RNA	A	61.20±1.06 (100)	55.10±0.58$^+$ (90)	45.90±0.78$^+$ (75)
	B	60.10±0.54 (100)	54.70±0.82$^+$ (91)	46.30±1.02$^+$ (77)
Protease	A	0.335±0.072 (100)	0.382±0.061$^+$ (114)	0.422±0.065$^+$ (126)
	B	0.328±0.062 (100)	0.367±0.052$^+$ (112)	0.407±0.082$^+$ (124)
Acid phosphatase	A	0.192±0.0003 (100)	0.172±0.0004$^+$ (89)	0.148±0.0007$^+$ (77)
	B	0.192±0.0002 (100)	0.175±0.0004$^+$ (91)	0.150±0.0006$^+$ (78)
Alkaline phosphatase	A	0.400±0.0010 (100)	0.366±0.0005$^+$ (92)	0.302±0.0005$^+$ (76)
	B	0.400±0.0005 (100)	0.372±0.0012$^+$ (93)	0.308±0.0003$^+$ (77)

Details are as given in Table I

Activity of acid phosphatase was inhibited to 89% and 77% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue of snail. Activity of alkaline phosphatase was reduced to 92% and 76% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in nervous tissue. Protease activity was increased to 114% and 126% of controls after treatment with 40% and 80% of LC_{50} (24h) of aqueous latex extracts respectively in the nervous tissue of snail *Lymnaea acuminata*. The maximum increase in protease activity (140% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous latex extract, respectively (Table III-IV).

Latex of *Alstonia scholaris* was sequentially extracted with organic solvents also caused a similar alteration in nitrogenous metabolism of snail. Alterations caused by sequentially extracted latex, though statistically in significant, appeared lower than the water extracted latex at all the doses and exposure periods (Table III-IV).

DISCUSSION

It is clear from the result described above that the treatment with sub-lethal doses of aqueous and partially purified latex extracts of *Thevetia peruviana* and *Alstonia scholaris*, significantly alter the level of total protein, total free amino

Table IV. Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg moles of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in nervous tissue of *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous and partially purified latex extracts of *Alstonia scholaris* for 96 h.

Tabla IV. Cambios en los niveles de proteína total, aminoácidos libres, ácidos nucléicos (DNA y RNA) (µg/mg), proteasa (µg moles de equivalentes de tirosina/mg proteína/h) y fosfatasas ácida y alcalina (µ moles sustrato hidrolizado/30 min/mg proteína) en el tejido nervioso de Lymnaea acuminata *tras 96 h de exposición a extractos acuosos y parcialmente purificados de latex de* Alstonia scholaris *al 40% y 80% de* LC_{50}.

	Nature of latex	Control	40% of LC50	80% of LC50
Protein	A	65.60±0.48 (100)	30.20±0.84* (46)	22.90±0.78* (35)
	B	66.20±0.58 (100)	31.80±0.96* (48)	23.80±0.48* (36)
Amino acid	A	35.60±0.76 (100)	51.60±0.88* (145)	56.90±0.78* (160)
	B	36.50±0.54 (100)	51.80±0.46* (142)	57.30±0.66* (157)
DNA	A	74.80±1.05 (100)	44.90±0.78* (60)	29.20±0.48* (39)
	B	75.60±1.08 (100)	46.90±0.78* (62)	31.00±1.02* (41)
RNA	A	60.10±0.74 (100)	36.10±0.74* (60)	27.00±0.48* (45)
	B	61.50±0.78 (100)	38.71±1.06* (63)	29.00±0.48* (47)
Protease	A	0.340±0.076 (100)	0.408±0.035* (120)	0.476±0.076* (140)
	B	0.338±0.066 (100)	0.399±0.067* (118)	0.463±0.064* (137)
Acid phosphatase	A	0.193±0.0005 (100)	0.094±0.0003* (49)	0.074±0.1133* (38)
	B	0.192±0.0008 (100)	0.099±0.0007* (52)	0.072±0.0006* (38)
Alkaline phosphatase	A	0.380±0.0007 (100)	0.188±0.0010* (49)	0.145±0.00038* (38)
	B	0.390±0.0007 (100)	0.195±0.0009* (50)	0.148±0.0004* (39)

Details are as given in Table I

acid and nucleic acid and activity of enzyme protease, acid and alkaline phosphatase. The rate of alteration in all the cases was significantly ($P<0.05$) time and dose dependent.

Proteins are mainly involved in architecture of the cell. During chronic periods of stress they are also a source of energy (UMMINGER, 1977). The decrease in protein level observed in this study may be due to their degradation and also to their possible utilization for metabolic purposes. BRADBURY, SYMONIC, COATS AND ATCHISON (1987) pointed out that the decrease protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The quantity of protein depends on the rate of protein synthesis or its degradation. It also affected due to impaired in corporation of amino acids in to polypeptide chains (SINGH *ET AL.*, 1996). The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcription level, thus may affect the protein level. In this study, a significant decline in RNA level in exposed snail was observed. The decrease in RNA level may also have been a cause of protein depletion. On the other hand, increase in protease activity may be the cause of increased protein degradation.

Enhanced protease activity and decreased protein level have resulted in marked elevation of free amino acids

that in the snail tissue. The accumulation of free amino acids can also be attributed to the lesser use of amino acids (Seshagiri Rao, Srinivas, Moorthy, Swamy and Chethy, 1987) and their involvement in the maintenance of an acid-base balance (Moorthy, Kashi Reddy, Swamy and Chethy, 1984). Natarajan (1985) reported that stress condition in general induces elevation in the trans-amination pathway.

Extracts of both the plants also decreased the level of nucleic acids significantly in the nervous tissue of the snail. Several reports are available on the reduction in DNA and RNA level on exposure to different pesticides (Tarig, Haqui and Adhami, 1977; Nordenskjold, Soderhall and Moldeus, 1979). Data attained in this study made it clear that these plant extracts are potential inhibitor of DNA synthesis, which resulted in the reduction in the RNA level. Mahendru (1981) suggested that the anti-AChE compounds attack many enzymes responsible for normal metabolism pathway. Thus it is possible that lattices of both the plants might have inhibited the enzymes necessary for DNA synthesis, because the lattices of both the plant tested in the present study have potent anti-AChE activity (Singh and Singh, 2002).

Vorbrodt (1959) has reported that alkaline phosphatase is an important enzyme of animal metabolism, which play an important role in the transport of metabolites a cross the membranes. Since, both the plants used in the present study may also have anti-phosphatases activity. So the reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (Pilo, Asnani and Shah, 1972) and also involved in the synthesis of certain enzymes (Sumner, 1965).

CONCLUSIONS

We therefore believe that these plant extracts may eventually be of great value for the control of aquatic target organisms, i.e. harmful vector snails and mosquito larvae.

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